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THE DEVELOPMENT OF THE ANDROGENIC GLANDS OF A PROTANDRIC SHRIMP [1]

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The protandric nature of the pandalid shrimps from the northwest coast of North America was first observed by Berkeley (1929, 1930). In these species of caridean decapods, the male stages possess hermaphroditic gonads, but only the testicular elements become functional. After one to three years, depending on the species of shrimp, the males transform into fully functional females with true ovaries. Charniaux-Cotton (1958) and Carlisle (1959a, 1959b, 1959c) reported that the onset of the female phase in *Pandalus borealis* coincides with the disappearance of a pair of endocrine organs, the androgenic glands. The role of the androgenic glands in talitrid amphipods is to control primary and secondary sexual differentiation and development through the secretion of an androgenic hormone (Charniaux-Cotton, 1952, 1954, 1955, 1957). Although data have been reported on the androgenic glands of the higher Crustacea (Charniaux-Cotton, Zerbib and Meusy, 1966), little is known concerning the development and differentiation of the androgenic glands of protandric decapods. One reason for this lack of information is the difficulty encountered in obtaining all of the developmental stages of a protandric shrimp on a seasonal basis.

The present report includes most of the developmental stages of the androgenic glands of the Pacific pandalid, *Pandalus platyceros* Brandt. In addition to the analysis of the development of the androgenic glands, correlations are made between structure and function.

Materials and Methods

Pandalus platyceros Brandt was collected on a monthly basis from June 1965 through January 1968. The shrimp were collected in Lopez Sound, Washington in fifteen to twenty-seven fathoms of water with a ten foot beam trawl. In the laboratory the shrimp were maintained in twenty to thirty gallon plexiglas aquaria that were supplied with running sea water. Generally the shrimp were sacrificed within a day or two of collecting. About 250 animals were utilized in this study.

The abdomen was excised with a razor blade and the carapace of the thorax was cut away to expose the gonads and sperm ducts. The ejaculatory bulbs and associated androgenic glands are located in the region where the sperm ducts are

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inserted on the sternal portion of the fifth walking legs. The androgenic glands were removed while still attached to the sperm ducts with fine forceps and iridectomy scissors and were immediately placed in fixatives.

Although Bouin's and Heidenhain's "Susa" fixatives were used initially for orientation purposes, a glutaraldehyde fixation was employed routinely thereafter. Primary fixation was in equal volumes of 5% aq solution of glutaraldehyde in 0.27 *M* NaCl and 0.4 *M* Millonig's phosphate buffer pH 7.4. After one hour fixation the tissue was rinsed in a solution of the phosphate buffer and 0.6 *M* NaCl (1:1 v/v). The tissue was then postfixed in an ice bath for one hour in the following solution: 1 part 4% osmium tetroxide; 1 part phosphate buffer; 2 parts 0.75 *M* NaCl. The osmolarity of all the fixatives and rinse was maintained at about 975 milliosmoles.

The tissue was dehydrated through increasing concentrations of ethanol and passed through three changes of propylene oxide to prepare the tissue for infiltration with Epon epoxy resin. It was then embedded in the resin according to the method of Luft (1961). Sections of one to two microns were cut on a Porter-Blum Ultratome using glass knives. The sections were stained on a hot plate using Richardson's stain (Richardson, Jarett and Finke, 1960), allowed to dry and then covered with Permount or Xam.

Results

The androgenic glands of *Pandalus platyceros* are located on the dorso-medial surfaces of the ejaculatory bulbs which in turn are at the distal ends of the sperm ducts (Fig. 1). The ejaculatory bulbs are highly muscular sphincters that allow the passage of the sperm strand through the male gonopore during copulation. The glands are composed of highly folded anastomosing cords of epithelial cells (Fig. 2). During the male phase each gland has a compact leaf-like appearance with the widest portion resting on the ejaculatory bulb and then extending up to the medial side of the sperm duct. From late spring through early summer the glands reach their maximum size of 1–2 mm in total length. The glands are supported on the sperm ducts by an abundance of connective tissue and small muscle filaments which appear to be derived from the musculature of the ejaculatory bulbs. No associated masses of androgenic tissue was found. Adipose tissue and lymphogenous nodules are located close to the androgenic glands. In paraffin embedded tissue the lymphogenous nodules may be confused with androgenic gland because of their similarity. However, from thick plastic sections and from numerous observations made from lymphogenous tissue in other regions of the shrimp, it is quite evident that this tissue is not androgenic gland tissue.

The early development and origin of the androgenic glands

The developmental state of the androgenic glands of *Panadalus platyceros* is a function of the size of the shrimp and not its age. Shrimp of the same age may vary considerably in size and state of development. In immature shrimp less than 2.2 cm CL (carapace length) which are six to eight months old (September through November), the muscular sheaths that encapsulate the epithelium of the sperm ducts have yet to be formed or are in the initial stages of being formed. A mass

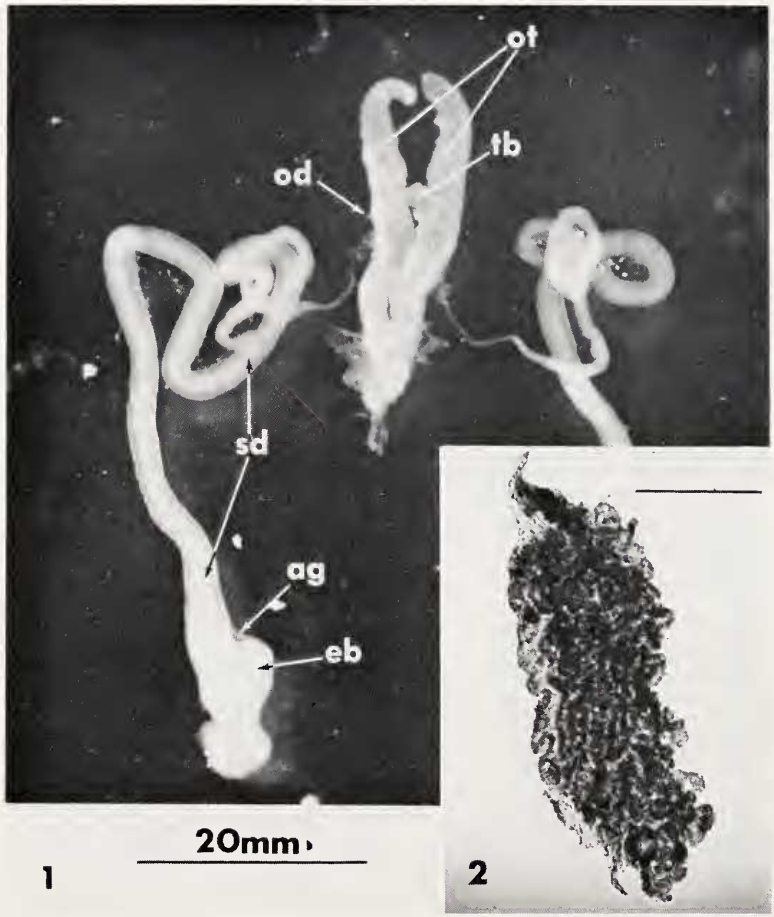

FIGURE 1. Reproductive system of a male *Pandalus platyceros* (3.5 cm carapace length, August 1967); Whole preparation in sea water: ag, androgenic gland, eb, ejaculatory bulb of sperm duct; od, oviduct; ot, ovotestis; sd, sperm duct; tb, transverse bridge of ovotestis.

FIGURE 2. Whole mount of an androgenic gland of a male *Pandalus platyceros* (2.9 cm carapace length, June 1967). The slash mark represents 0.5 mm.

of cells can be seen at the dorsal surface of each ejaculatory bulb (Fig. 3), and appear to be the early androgenic glands. In fact, peripheral portions of these cell masses can be seen in cord-like arrays. In immature males larger than 2.2 cm CL, the androgenic glands resemble the adult glands, but they are composed of very few cords of cells. The epithelial linings of the sperm ducts have now developed a muscular sheath. From transverse sections through the distal ends of the differentiating ejaculatory bulbs, it is apparent that androgenic gland cells are proliferating from cells in the ejaculatory bulb epithelium (Fig. 4). These newly formed rectilinear arrays of tissue have moved between the muscle masses that encapsulate this region (Figs. 5 and 6). No mitotic figures are evident in this intermuscular androgenic tissue. The most peripheral muscle bands of the ejacula-

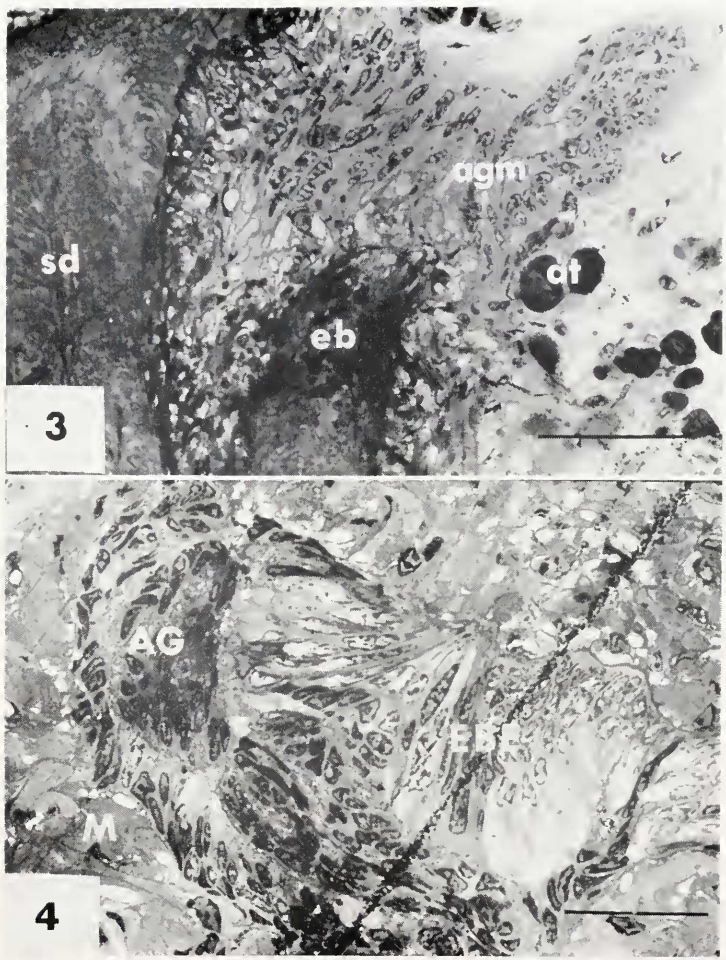

FIGURE 3. Transverse section (1 μ) of the androgenic gland mass from an immature male *P. platyceros* (1.7 cm carapace length, September); Glutaraldehyde-Osmium fixation, Richardson's stain. The slash mark represents 50 microns; agm, androgenic gland mass; at, adipose tissue; eb, ejaculatory bulb epithelium; sd, sperm duct epithelium.

FIGURE 4. Transverse section (1 μ) through the dorsal region of the ejaculatory bulb of an immature male (2.2 cm carapace length, November) showing the relationship between the androgenic gland tissue (AG) and the epithelium of the ejaculatory bulb (EBE); Glutaraldehyde-Osmium fixation, Richardson's stain. The slash mark represents 25 microns, M, musculature of the ejaculatory bulb.

tory bulb appear to disassociate from each other, liberating the glandular tissue into the hemocoel. This phenomenon may account for the muscle bands that are found in loose association with some of the androgenic cords in the adult male stage.

Although the androgenic gland cells appear to originate from the differentiating epithelium of the ejaculatory bulb, actual cell division and active growth occur during the winter and spring months in the androgenic cords that lie free in the hemocoel.

In reproductive males that have sperm ducts greatly distended with sperm, the sperm duct epithelium produces a secretion stained with Orange G that forms the sheath of the sperm strand (Fig. 7). At this stage of development no intermuscular masses of androgenic tissue are evident within the musculature of the ejaculatory bulb, and the androgenic glands have lost their connection with the musculature. In contrast, males which have not produced sperm during the reproductive season still show intermuscular masses of androgenic tissue (Fig. 6) and the androgenic glands are connected with the musculature of the sperm ducts.

Carlisle (1959a) reports the androgenic glands of *Pandalus borealis* attached to a strip of vas deferens (sperm duct) epithelium. It is difficult to state for certain for his drawings of this connection are rather diagrammatic, but this epithelial layer may be part of the ejaculatory bulb. Charniaux-Cotton *et al.* (1966) have not seen this connection in the same species of shrimp. These two investigators may be reconciled if one considers that the glands of *P. platyceros* lose their connection with the musculature during the sexually active male stage. Thus the investigators may be describing different stages of development.

There is a striking difference in the morphology of the sperm duct epithelium between reproductive and nonreproductive males. In nonreproductive males, the lumina of the sperm ducts may or may not have become patent and the epithelial layer contains tall columnal, highly compact epithelial cells (Fig. 8). However, in reproductive males, the height of the epithelium has been reduced and its cytoplasmic affinities for basic stains have nearly disappeared (Fig. 7).

The developmental cytology of the androgenic gland cells

The androgenic gland cells of *Pandalus platyceros* generally contain ovoid to discoidal nuclei measuring on the average fifteen microns along their long axis. The shape of the nucleus is related to the number of cells per unit length of androgenic cord. When the number of cells per unit length is small, the nuclei tend to be more ovoid in appearance. When the number of cells per unit length is large, the cells are highly compressed against each other very much like overlapping shingles. This results in extremely flat, discoidal nuclei. The general irregular outline of the intermuscular androgenic gland cell nucleus probably is due to mechanical pressure from the muscle bands. The cytoplasm of the gland cells generally has affinities for basic stains, although there is considerable variation in stainability from cell to cell. No histologically stainable cell product is ever evident in any of the cells, although acid phosphatase positive granules (Gomori, 1950) are present in many of the cells.

The developmental histology of the androgenic cells of *P. platyceros* can be divided into six discreet stages (Fig. 9).

Stage 1 or the stage of proliferation is typical of immature androgenic cord cells and intermuscular cells. This stage is characterized by cells with relatively small cytoplasmic volume, small irregularly shaped nuclei with a diameter of about ten microns and, in the cord cells only, an abundance of mitotic figures.

Stage 2 or the stage of cytoplasmic hypertrophy is found during the winter, spring and early summer months at the time of the active male phase. During these times of the year the gonads are actively proliferating spermatogonia and a relatively small number of oogonia. This stage is characterized by an elongation of the long axis of the cells to 50 μ in length; a flattening and lengthening of the nuclei

into ovoids or discs; the presence of dense chromatin granules and ribbons in the nucleoplasm; an increase in the affinity of the cytoplasm for basic stains; an increase in cytoplasmic volume; the presence in the cytoplasm of Gomori acid phosphatase positive granules; and a thinning of the androgenic sheath to a thickness of one or two microns. This stage of development is characteristic of most of the cells that comprise the androgenic glands proper and may be considered to represent the typical androgenic gland cells of *Pandalus platyceros*.

Stage 3 or the stage of vacuolization is found during the winter, spring and early summer months at the time of the active male phase, but the total number of vacuolated cells comprise only a small number of the total number of gland cells. This stage appears to reach a peak during the early summer months. They are not localized in any specific region of the gland but are scattered in clusters throughout the gland. The morphological characteristics of this stage are the gradual loss of affinity by the nuclei for basic stains and the swelling of the nuclei into ovoid form; the loss of affinity of the cytoplasm for basic stains; the disappearance of the Gomori acid phosphatase positive granules from the cytoplasm; and the formation in the cytoplasm of vacuoles which do not stain in any characteristic way.

Stage 4 or the stage of cellular breakdown closely follows the preceding stage and is always found in association with stage 3. This stage is characterized by enlarged nuclei that have almost completely lost their affinity for basic stains, and by the total degeneration or break-up of the cytoplasm. At times, such large swollen nuclei can be found intact in the hemocoel near the androgenic cords.

Stage 5 or the stage of transitional atrophy is found in all of the androgenic gland cells of transitional males during the late summer and early autumn months. Also such cells are found in the peripheral regions of the androgenic glands of nontransforming (nonreproductive) males during the same times of the year. This stage is characterized by irregular and densely staining pycnotic nuclei, and diminution of the long axis of the cells, the presence of large cytoplasmic inclusions that range up to six microns in length, and a thickening and folding of the androgenic sheath.

Stage 6 or the ghost stage is characterized by empty sheaths of androgenic cords. It occurs in the latter months of autmn and early winter. The entire androgenic glands of transforming males is typified by this stage. The gonads of these shrimp totally lack testicular elements and contain only ovarian cells. Androgenic glands of nontransforming males contain many ghost areas in the periphery of the gland, but also show new cords regenerating from the intermuscular cells (stage 1). This stage is characterized by empty sheaths of androgenic cords or "ghosts" of androgenic gland cells in which pycnotic nuclei are sometimes evident.

Discussion

The observations that the androgenic glands of the protandric hermaphrodite, *Pandalus platyceros* originate from the epithelium of the sperm duct that will differentiate into the ejaculatory bulb corroborate the results of previous workers who have given a genital origin for the androgenic glands of the higher Crustacea (Charniaux-Cotton *et al.*, 1966). The sperm ducts and associated ejaculatory bulbs originate from the posterior extensions of the early gonadal tube (Hoffman, 1968). Any derivative from the epithelium that lines these tubes would be of gonadal origin. Demeusy (1960) noted in the postembryonic development of the androgenic glands

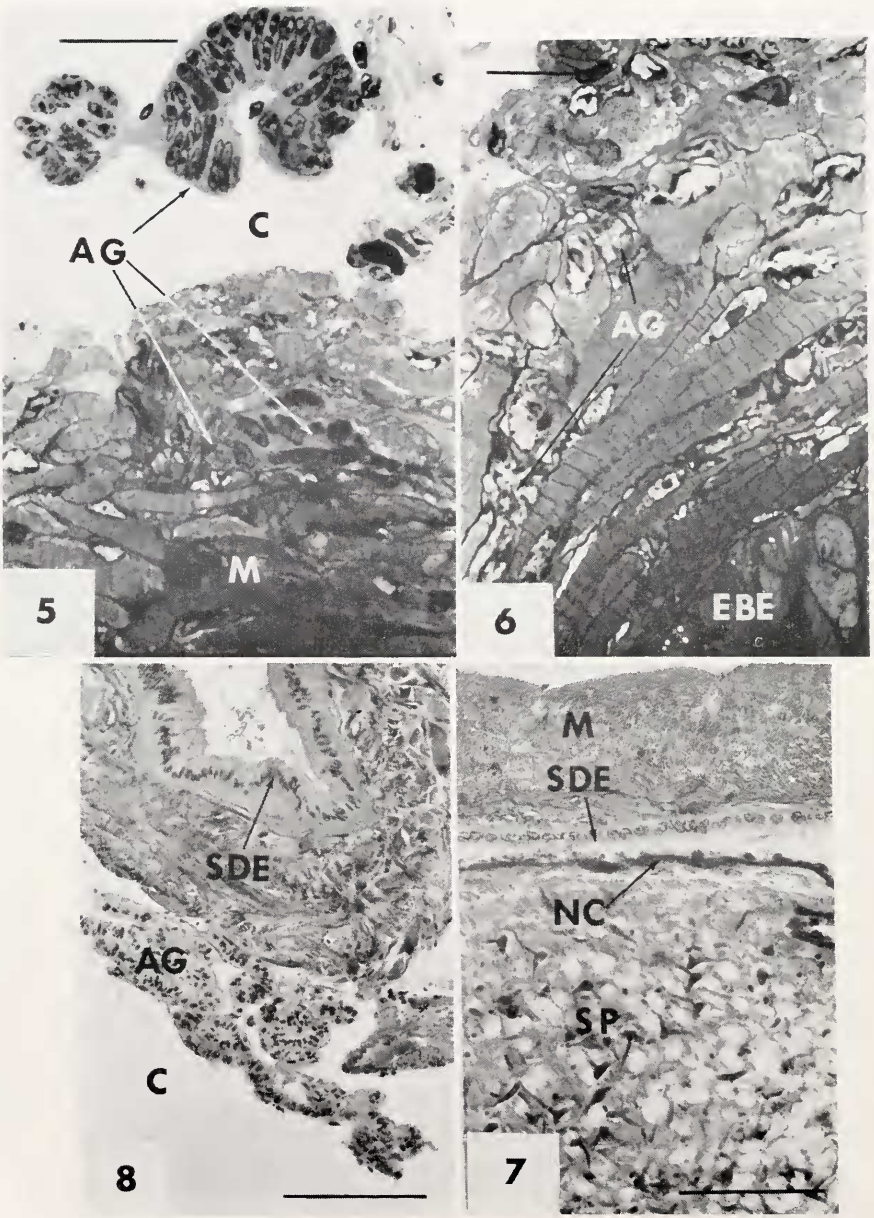

FIGURE 5. Transverse section (1 μ) through the musculature of the ejaculatory bulb showing androgenic gland cells between the muscle bands (2.4 cm carapace length, December); Glutaraldehyde-Osmium fixation, Richardson's stain. The slash mark represents 25 microns; AG, androgenic gland cells; C, hemocoel; M, musculature of the ejaculatory bulb.

FIGURE 6. Transverse section (1 μ) through the musculature of the ejaculatory bulb showing androgenic gland cells between the muscle bands (nonreproductive male, 3.0 cm

of *Carcinus maenas* that small intermuscular masses of androgenic tissue are present by the sperm ducts, but these masses were not related to the epithelium of the sperm ducts proper.

It is apparent that the androgenic glands of this protandric shrimp disappear because the source of these cells has completely differentiated into an ejaculatory bulb epithelium. Therefore this epithelial layer has lost the ability to differentiate into androgenic gland cells.

Following spermiogenesis, when the sperm ducts have become greatly distended with sperm and spermatic fluid, the tall columnar epithelial cells of the sperm ducts secrete a sheath that forms the sperm into long transparent strands or filaments. These epithelial cells then appear to regress, but do not completely degenerate until several months later. This change in function and morphology coincides with the disappearance of the intermuscular androgenic gland cells. At this time, the epithelium may have lost its capacity to differentiate into androgenic gland cells and sperm sheath producing cells. Before sperm was present in the ducts, the epithelium was in an "undifferentiated" state. In a study of the reproductive cycle of *Pandalus platyceros*, it has been shown that most if not all the males that become reproductive generally transform into females, while those that do not become reproductive generally remain in the male phase for another year (Hoffman, 1968). They then become reproductive and soon afterward transform into females.

The rectilinear arrays of androgenic tissue between the muscle bands of the differentiating ejaculatory bulbs may explain the morphology of the adult glands. As young cords of tissue work their way out of their muscular enclosure, cell divisions become evident. At this time the cells grow in length and become associated with each other into discreet glands. The gland proper is attached to the dorsal surface of each ejaculatory bulb during the male phase. It appears then that the replacement zone is located in this region of the sperm duct. Numerous observations show that the intermuscular androgenic masses find passage out of the muscle bands that envelope this region of the duct, but it is not known what physiological or mechanical mechanisms occur in the muscles to permit this release. Since numerous loose muscle bands are sometimes evident in or alongside the androgenic glands, they are thought to originate from the musculature of the ejaculatory bulb.

The androgenic glands have been reported to secrete in a holocrine manner (Charniaux-Cotton, 1960; Charniaux-Cotton and Kleinholz, 1963; Tcholakian and Reichard, 1964) on the basis of observations on the number of degenerating regions of the glands at times when they are considered to be functional. Yet no histological or histochemical products have ever been reported in any androgenic gland cell (Charniaux-Cotton *et al.*, 1966). No secretory product appears to be present in

carapace length, August); Glutaraldehyde-Osmium fixation, Richardson's stain. The slash mark represents 10 microns; AG, androgenic gland cells; EBE, ejaculatory bulb epithelium.

FIGURE 7. Transverse paraffin section (6 μ) through the sperm duct of a postreproductive transforming male (3.6 cm carapace length, November), Hematoxylin-Halmi stain. The slash mark represents 50 microns; M, musculature of sperm duct; NC, noncellular sheath of sperm strand; SDE, sperm duct epithelium; SP, sperm.

FIGURE 8. Transverse paraffin section (6 μ) through the sperm duct and attached androgenic gland (2.8 cm carapace length, July), Hematoxylin-Halmi stain. The slash mark represents 100 microns; AG, androgenic gland; C, hemocoel; SDE, sperm duct epithelium.

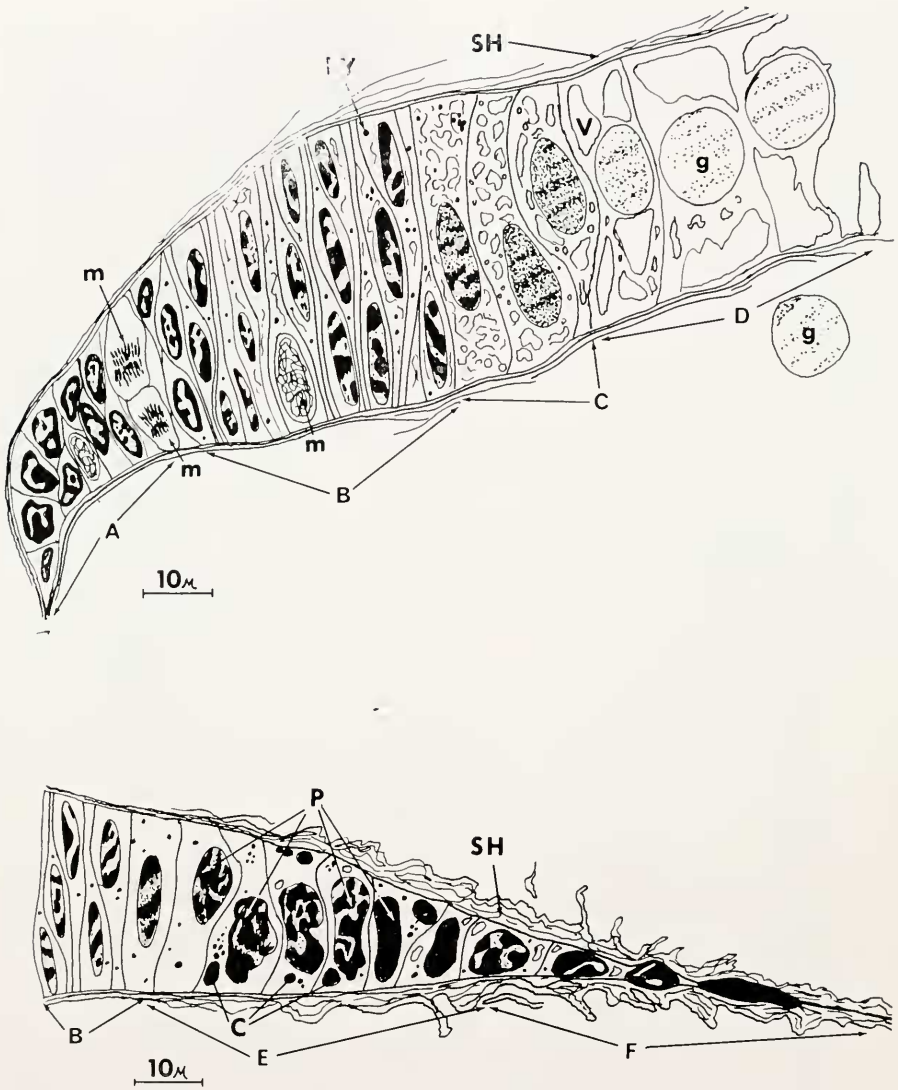

FIGURE 9. The developmental stages of the androgenic gland cells of *Pandalus platyceros*, A. Stage 1 or the stage of proliferation; B. Stage 2 or the stage of cytoplasmic hypertrophy; C. Stage 3 or the stage of vacuolization; D. Stage 4 or the stage of cellular breakdown; E. Stage 5 or the stage of transitional atrophy; F. Stage 6 or the ghost stage. C, cytolysosomes of Novikoff?; g, giant nuclei; Ly lysosomes?; m, mitotic nuclei; P. pycnotic nuclei; SH, sheath of androgenic cords.

any of the androgenic gland cells of *P. platyceros*. King (1964) has described multivesicular bodies in the androgenic gland cells of *Pachygrapsus crassipes* and these Gomori acid phosphatase bodies appear to be lysosomes. Similar inclusions have been found in the gland cells of *P. platyceros*, especially in stage 2 but absent in subsequent stages. Novikoff (1961) has stated that lysosomes are not the

causative agents of cell death, but become activated releasing their autolytic enzymes after the cell has already died. Perhaps the stage 3 cells are in the initial stages of cell death since the lysosome-like bodies are no longer evident.

The degenerating androgenic glands of transforming *P. platyceros* males do not at all resemble the degenerative cells described by Charnaiux-Cotton (1960) as representative of holocrine secretion. No vacuolar cells are present in the glands during the transformation to the female phase. The nuclei do become pycnotic, but the cytoplasm stains intensely with basic stains and numerous large inclusions are evident within the cells. These large inclusions so typical of stage 5 may be similar to the large lysosomes or cytolysosomes described by Novikoff (1959, 1960) in dying liver cells and dying cells of atretic ovarian follicles. Scharrer (1966) has reported large irregular membrane bound bodies up to 4 μ in diameter within the degenerating prothoracic gland cells of *Leucophaea* and *Blaberus* a few days after the final molt. Scharrer believes that these bodies represent autophagic vacuoles.

Further evidence opposing or questioning holocrine secretion as the cause of the degeneration seen in stage 5 is found in the gonad, the major target organ of the androgenic hormone. When the cells are at stages 5 and 6 in late summer and early autumn, the gonad has ceased to elaborate new spermatogonia from the germinal epithelium. By November the androgenic glands have disappeared in transforming males and the gonad has become a true ovary (Hoffman, 1968). The disappearance of the androgenic glands of *Pandalus platyceros* and thus the initiation of the female phase appear to be under some form of inhibitory control from the eyestalk complex (Hoffman, 1968b).

The development of the androgenic gland cells of *P. platyceros* may demonstrate merocrine as well as holocrine activity. In this case the stage 2 cells could be releasing their product in a merocrine manner. The degeneration that is evident in stages 3 and 4 then would be indicative of cell death. In addition nothing significant is known concerning the precise chemical nature of the androgenic hormone due to the lack of a reliable bioassay. Therefore, the mechanism of secretion of the androgenic hormone remains obscure despite previous claims of holocrine activity.

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Summary

1. The androgenic glands of the protandric shrimp, *Pandalus platyceros*, are shown to originate from a region of the sperm duct epithelium that will later differentiate into the ejaculatory bulbs.

2. The disappearance of the androgenic glands at the time of sex transformation appear to be related to gonadal function and the secretory activity of the sperm duct epithelium.

3. A developmental scheme is presented for the androgenic gland cells of *P. platyceros*. The cells are classified into six developmental stages.

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